FORM PTO-1390 — U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

Attorney's Docket No.: 1871-129

U.S. Application No. (if known, see 37 CFR 1.5): 09/509196

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/AU98/00795	23 September 1998	23 September 1997

TITLE OF INVENTION
A Potential Effector for the Grb7 Family of Signalling Proteins

APPLICANT(S) FOR DO/EO/US
Roger John DALY, Robert Lyndsay SUTHERLAND

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US)
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has **NOT** expired.
   d. [X] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

ITEMS 11. TO 16. below concern other document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [X] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A **FIRST** preliminary amendment.
    [ ] A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:
    - Copy of published application 99/15647
    - Copy of International Preliminary Examination Report
    - Sequence Listing in computer readable format

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) 09/509196	INTERNATIONAL APPLICATION NO. PCT/AU98/00795	ATTORNEY DOCKET NO. 1871-129

17. [X] The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492)(a)(1)-(5):		
Search Report has been prepared by the EPO or JPO	$ 840.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482)	$ 670.00	
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))	$ 690.00	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$ 970.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)	$ 96.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 970.00	
Surcharge of $130.00 for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	18 -20 =	0	X $18.00	$ 0	
Independent Claims	2 - 3 =	0	X $78.00	$ 0	
Multiple dependent claim(s) (if applicable)			+ $260.00	$ 0	
TOTAL OF ABOVE CALCULATIONS =				$ 970.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$ 970.00	
Processing fee of $130.00 for furnishing the English translation later [ ] 20 [ ] 30 than months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 970.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$ 40.00	
TOTAL FEES ENCLOSED =				$ 1,010.00	
				Amount to be refunded	$
				charged	$

a. [X] Checks in the amount of $ 970.00 and 40.00 to cover the above fees are enclosed.

b. [ ] Please charge my Deposit Account No. 02-2135 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-2135. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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Name

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Registration Number

1871-129
EE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
	)	
Roger J. DALY et al.	)	Filing Under 35 USC 371
	)	International Application
Serial No.	)	No. PCT/AU98/00795
	)	Filed: 23 September 1998
Filed:	)	
	)	
For: A POTENTIAL EFFECTOR FOR THE GRB7 FAMILY OF SIGNALLING PROTEINS	)	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to calculating the filing fee for the above-referenced patent application, please enter the following amendments:

In the Claims:

In claim 5, line 2, please delete "any one of the preceding claims" and insert therefor --claim 1--.

In claim 7, line 2, please delete "or 6".

In claim 8, line 2, please delete "any one of claims 1 to 4" and insert therefor --claim 1--.

In claim 11, line 2, please delete "or 9".

In claim 12, line 4, please delete "any one of claims 1 to 4" and insert therefor --claim 1--.

In claim 15, line 3, please delete "or 13".

Please add the following new claims:

16. An antibody or fragment thereof which specifically binds to a protein according to claim 9.

17. A method of detecting in a sample the presence of an effector protein for the Grb7 family of proteins, the method comprising reacting the sample with an antibody or fragment thereof according to claim 16.

18. A method of detecting in a sample the presence of mRNA encoding an effector protein for the Grb7 family of proteins, the method comprising reacting the sample with an oligonucleotide probe of claim 13.

REMARKS

The amendments set forth above are made to simplify the claim dependencies. No new matter is introduced into the application by means of these amendments.

Respectfully submitted,

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1871-129.PRE

A POTENTIAL EFFECTOR FOR THE GRB7 FAMILY OF SIGNALLING PROTEINS

Field of the Invention:

The present invention relates to a novel polynucleotide molecule encoding a candidate effector protein for the Grb7 family of signalling proteins. Detection of the encoded protein in a tissue sample should provide a useful tumour marker and/or prognostic indicator. Furthermore, antagonism of the interaction between Grb7 family members and the encoded protein should provide a novel treatment strategy for human diseases exhibiting aberrant receptor tyrosine kinase (RTK) signalling (e.g. cancer).

Background of the Invention

RTKs play a major role in the regulation of cellular growth, differentiation, motility and metabolism by converting an extracellular signal in the form of the binding of a specific hormone or growth factor to the activation of specific signalling pathways and hence modes of intracellular communication (Schlessinger and Ullrich, *Neuron* 9, 383-391, 1992). Activation of RTKs results in both autophosphorylation of the receptor and the phosphorylation of downstream targets on tyrosine residues. It has become evident over the last decade that key elements in receptor-substrate and other protein-protein interactions in RTK signalling are src homology (SH)2 domains. SH2 domains are conserved modules of approximately 100 amino acids found in a wide variety of signalling molecules which bind to short tyrosine-phosphorylated peptide sequences. The specificity of interaction is determined both by the nature of the amino acids flanking the phosphotyrosine residue in the target peptide and residues in the SH2 domain which interact with these sites (Pawson, *Nature* 373, 573-580, 1995).

SH2-domain containing proteins can be divided into two classes: those which possess a catalytic function (e.g. the cytoplasmic tyrosine kinase c-src and the tyrosine phosphatase SH-PTP2) and those which consist entirely of non-catalytic protein domains (eg Grb2), the adaptor sub-class. The function of the latter class is to link separate catalytic subunits to a tyrosine-

phosphorylated receptor or signalling intermediate, and other non-catalytic protein modules are often involved in these interactions. For example, SH3 and WW domains (conserved regions of approximately 50 and 40 amino acids, respectively) bind proline-rich peptide ligands, and pleckstrin homology domains (approximately 100 amino acids) interact with both specific phospholipid and protein targets (Pawson, 1995 *supra*).

The Grb7 family represents a family of SH2 domain-containing adaptors which currently contains three members: Grb7, 10 and 14 (Margolis *et al, Proc. Natl. Acad. Sci. USA* 89, 8894-8898, 1992; Stein *et al, EMBO J* 13, 1331-1340, 1994; Ooi *et al, Oncogene* 10, 1621-1630, 1995; Daly *et al, J. Biol. Chem.* 271, 12502-12510, 1996). These proteins share a common overall architecture, consisting of an N-terminal region containing a highly conserved proline-rich decapeptide motif, a central region harbouring a PH domain and a C-terminal SH2 domain. The central region of approximately 300 amino acids bears significant homology to the *C. elegans* protein mig10, which is required for long range neuronal migration in embryos, otherwise the Grb7 family and mig10 are structurally distinct. However, they exhibit differences in both SH2 selectivity towards RTKs (Janes *et al, J. Biol. Chem.* 272, 8490-8497, 1997) and tissue distribution. The family has therefore evolved to link particular receptors to downstream effectors in a tissue-specific manner. Interestingly, the genes encoding this family appear to have co-segregated with *ERBB* family genes during evolution. Thus *GRB7, 10* and *14* are linked to *ERBB2, ERBB1* (epidermal growth factor receptor) and *ERBB4*, respectively (Stein *et al* 1994 *supra*; Ooi *et al*, 1995 *supra*; Baker *et al*, *Genomics* 36, 218-220, 1996). The juxtaposition of *GRB7* and *ERBB2* leads to common co-amplification in human breast cancers, and since the two gene products are functionally linked, likely up-regulation of an undefined erbB2 signalling pathway. Furthermore, *GRB14* also exhibits differential expression in human breast cancers (Daly *et al*, 1996 *supra*). These two proteins may therefore modulate RTK signalling in this disease.

In order to identify proteins which bind to this family and therefore identify candidate effectors, we performed a genetic screen using the yeast two hybrid system and Grb14 "bait". This application describes the cloning and characterization of a novel interacting protein, currently designated 2.2412.

Disclosure of the Invention:

Thus, in a first aspect, the present invention provides an isolated polynucleotide molecule encoding a candidate effector protein for the Grb7 family of signalling proteins, wherein the polynucleotide molecule comprises a nucleotide sequence having at least 75% sequence identity to that shown as SEQ ID NO: 1.

Preferably, the polynucleotide molecule comprises a nucleotide sequence having at least 85%, more preferably at least 95%, sequence identity to that shown as SEQ ID NO: 1. Most preferably, the polynucleotide molecule comprises a nucleotide sequence encoding a polypeptide comprising an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 2.

In a preferred embodiment of the invention of the first aspect, the polynucleotide molecule comprises a nucleotide sequence which substantially corresponds to that shown as SEQ ID NO: 1.

The polynucleotide molecule may be a dominant negative mutant which encodes a gene product causing an altered phenotype by, for example, reducing or eliminating the activity of endogenous effector proteins of the Grb7 family of signalling proteins.

The polynucleotide molecule may be incorporated into plasmids or expression vectors (including viral vectors), which may then be introduced into suitable host cells such as bacterial, yeast, insect and mammalian host cells. Such host cells may be used to express the protein encoded by the polynucleotide molecule.

Accordingly, in a second aspect, the present invention provides a host cell transformed with the polynucleotide molecule of the first aspect.

In a third aspect, the present invention provides a method of producing a protein, comprising culturing the host cell of the second aspect under conditions suitable for the expression of the polynucleotide molecule and optionally recovering the protein.

Preferably, the host cell is mammalian or of insect origin. Where the cell is mammalian, it is presently preferred that it be a Chinese hamster ovary (CHO) cell or human embryonic kidney (HEK) 293 cell. Where the host cell is of insect origin, it is presently preferred that it be an insect Sf9 cell.

In a fourth aspect, the present invention provides a purified protein encoded by the polynucleotide molecule of the first aspect.

In a preferred embodiment of this aspect, the purified protein comprises an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 2.

In a fifth aspect, the present invention provides a fusion protein comprising an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 2.

Fusion proteins according to the fifth aspect may include an N-terminal fragment of a protein such as β-galactosidase to assist in the expression and selection of host cells expressing candidate effector protein, or may include a functional fragment of any other suitable protein to confer additional activity(ies).

In a sixth aspect, the present invention provides an antibody or fragment thereof which specifically binds to the protein of the fourth aspect.

The antibody may be monoclonal or polyclonal, however, it is presently preferred that the antibody is a monoclonal antibody. Suitable antibody fragments include Fab, $F(ab')_2$ and scFv.

In a seventh aspect, the present invention provides an oligonucleotide probe comprising a nucleotide sequence of at least 12 nucleotides, the oligonucleotide probe comprising a nucleotide sequence such that the oligonucleotide probe selectively hybridises to the polynucleotide molecule of the first aspect under high stringency conditions (Sambrook *et al.*, *Molecular Cloning: a Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press).

In a preferred embodiment of this aspect, the oligonucleotide probe is labelled. In a further preferred embodiment of this aspect, the oligonucleotide probe comprises a nucleotide sequence of at least 18 nucleotides.

In an eighth aspect, the present invention provides a method of detecting in a sample the presence of an effector protein for the Grb7 family of proteins, the method comprising reacting the sample with an antibody or fragment thereof the sixth aspect, and detecting the binding of the antibody or fragment thereof.

The method of the eighth aspect may be conducted using any immunoassays well known in the art (e.g. ELISA). The sample may be, for example, a cell lysate or homogenate prepared from a tissue biopsy.

In a ninth aspect, the present invention provides a method of detecting in a sample the presence of mRNA encoding an effector protein for the Grb7 family of proteins, the method comprising reacting the sample with an oligonucleotide probe of the seventh aspect, and detecting the binding of the probe.

The method of the ninth aspect may be conducted using any hybridisation assays well known in the art (e.g. Northern blot). The sample may be a poly(A) RNA preparation or homogenate prepared from a tissue biopsy.

Grb7 family proteins exhibit differential expression in certain human cancers (particularly breast and prostate cancer) and may therefore be involved in tumour progression. Detection of the protein encoded by the cDNA 2.2412 in a sample should provide a useful tumour marker and/or prognostic indicator for these cancers. Furthermore, the interaction of Grb7 family members with 2.2412 may provide a novel target for therapeutic intervention.

It is to be understood that methods of detecting suitable agonists and methods of therapy utilising detected agonists also form part of the present invention.

The term "substantially corresponds" as used herein in relation to the nucleotide sequence shown as SEQ ID NO: 1 is intended to encompass minor variations in the nucleotide sequence which due to degeneracy in the DNA code do not result in a change in the encoded protein. Further, this term is intended to encompass other minor variations in the sequence which may be required to enhance expression in a particular system but in which the variations do not result in a decrease in biological activity of the encoded protein.

The term "substantially corresponding" as used herein in relation to the amino acid sequences shown as SEQ ID NO: 2 is intended to encompass minor variations in the amino acid sequences which do not result in a decrease in biological activity of the protein. These variations may include conservative amino acid substitutions. The substitutions envisaged are:-

G, A, V, I, L, M; D, E; N, Q; S, T; K, R, H; F, Y, W, H; and

P. Nα-alkalamino acids.

The terms "comprise", "comprises" and "comprising" as used throughout the specification are intended to refer to the inclusion of a stated step. component or feature of group of steps. components of features with or without the inclusion of a further step. component or feature or group of steps. components or features.

The invention will hereinafter be described with reference to the accompanying figure and the following. non-limiting example.

Brief description of the accompanying figure:

Figure 1 provides the nucleotide and amino acid (single letter code) sequence of 2.2412. Numbers refer to distances in base pairs. Ankyrin-type repeat sequences are underlined. An additional repeat sequence is indicated by italics. The stop codon is represented by an asterisk. The original cDNA clone 2.2412 isolated by the two hybrid screen spans nucleotides 694-2664 of this sequence.

Figure 2 provides a map of the 2.2412-binding region on Grb14.
A. Structure of the deletion constructs used in the analysis. Gal4 DNA-BD fusion constructs encoding full length Grb14 (FL). the N-terminal (N). central region (C) and N-terminal + central region (N + C) were generated in the vector pAS2.1.
B. Results of β-galactosidase activity assays following transformation of the above plasmids into yeast strain Y190 together with the original 2.2412 cDNA clone in pACT-2.

Example: ***CLONING AND CHARACTERISATION OF 2.2412***

Yeast two hybrid screen

The yeast two hybrid system exploits protein-protein interactions to reconstitute a functional transcriptional activator which can then be detected using a gene reporter system (Fields and Sternglanz. *TIG.* 10. 286-292. 1994). The technique takes advantage of the properties of the Gal4 protein of the yeast *S. cerevisiae*. The Gal4 DNA binding domain (DNA-BD) or activation domain (AD) alone are incapable of inducing transcription. However, an interaction between two proteins synthesized as DNA-BD- and AD-fusions. respectively, brings the Gal4 domains into close proximity and results in

transcriptional activation of two reporter genes (*HIS3* and *LacZ*) which can be monitored by growth on selective medium and biochemical assays.

A plasmid construct encoding a Gal4 DNA-BD-Grb14 fusion was generated as follows. The plasmid *GRB14*/pRcCMVF containing full length *GRB14* cDNA (Daly *et al.* 1996) was restricted with HindIII and Klenow treated to create blunt ends. and then digested with *BclI* to release three fragments of approximately 1.1, 4.2 and 1.7 kb. The 1.7 kb fragment was isolated and cloned into the *NdeI* (Klenow treated) and *Bam*HI sites of the yeast expression vector pAS2.1 (Clontech) to generate *GRB14*/pAS2.1 containing an in-frame fusion of full length Grb14 with the GAL4 DNA-BD. This construct was introduced by electroporation into the yeast strain CG1945 (*MATa, ura3-52, his3-200, ade2-101, lys2-801,trp1-901, leu2-3, 112, gal4-542, gal80-538, cyh*^{r}*2, LYS2::GAL1*$_{UAS}$*-GAL1*$_{TATA}$*-HIS3, URA3::GAL417mers(x3)-CYC1*$_{TATA}$*-lacZ*) selecting for tryptophan prototrophy. The expression of the fusion protein was verified by Western blot analysis with antibodies directed against the Flag epitope and the Gal4 DNA-BD. The recipient strain was then grown to mid-log phase and a human liver cDNA library in the vector pACT2 (Clontech) introduced using the LiAc procedure (Schiestl and Gietz, *Curr. Genet.* 16. 339-346. 1989). Transformants were then selected for tryptophan, leucine and histidine prototrophy in the presence of 5mM 3-aminotriazole.

From a screen of $1x10^6$ clones, 39 colonies were initially selected on synthetic complete (SC)-leu-his-trp +3AT medium and were then tested for β-galactosidase activity. 12 clones scored positive in the latter assay and were subjected to cycloheximide (CHX) curing to remove the bait plasmid by streaking out on SC-leu media containing 10ug/ml CHX (pAS2-1 contains the *CYH2* gene which restores CHX sensitivity to CG1945 cells). This enabled confirmation of the bait dependency of *LacZ* activation and subsequent isolation of the pACT2 plasmids encoding interacting proteins by standard methodology (Philippsen *et al*, *Methods in Enzymology* 194, 170-177). Back transformations were then performed in which these pACT2 plasmids were introduced into CG1945 strains containing the bait plasmid (*GRB14*/pAS2-1) or constructs encoding non-related Gal4 DNA-BD fusions in order to confirm the specificity of the interactions.

The DNA sequences of the cDNA inserts were then obtained by cycle sequencing (f-mol kit. Promega) using pACT2-specific and/or clone-specific

primers. Based on their nucleotide sequences the 12 interacting clones were classified into 6 independent groups (see Table I).

TABLE I: Characterization of cDNA clones isolated by the yeast two hybrid screen.

Class	No. of clones	Identity	Mean RLU (Liquid assay)	Colour intensity (Filter assay)
1	6	Nedd4	2.86×10^6	++++
2	2	Htk	1.86×10^5	++
3	1	2.2412	5.18×10^6	++++
4	1	Proteosome	3.88×10^2	+/-
5	1	Somatostatin receptor	1.45×10^3	+/-
6	1	L-arginine:glycine amidinotransferase	8.61×10^2	+/-

The 12 clones exhibiting activation of both the *HIS3* and *lacZ* reporter genes were divided into 6 groups by sequence analysis of their cDNA inserts. Results of β-galactosidase activity assays performed using two methodologies are shown. The liquid culture-derived method (Galacto-Light, TROPIX) is more quantitative; results are given in mean relative light units (RLU) and are normalized for the protein content of the samples. Blue/white screening of the cDNA clones was also performed using a colony lift filter assay (Clontech). The intensity of blue colour development over approximately 2h is scored from +/- (very weak) to ++++ (strong).

Six clones were partial cDNAs corresponding to Nedd4, a multidomain protein containing a calcium-dependent phospholipid binding (CaLB) domain, four WW domains and a C-terminal region homologous to the E6-AP carboxyl-terminus (Kumar *et al*, *Biochem. Biophys. Res. Commun.* 185, 1155-1161, 1992; Sudol *et al J. Biol. Chem.* 270, 14733-14741, 1995; Huibregtse *et al Proc. Natl. Acad. Sci. USA* 92, 2563-2567, 1995). The latter is likely to confer E3 ubiquitin-protein ligase activity on Nedd4. The pACT2 clones isolated encoded the CaLB domain together with the first 22 amino acids of the first WW domain.

Two clones encoded the intracellular region and part of the extracellular domain of Htk, which is a RTK of the Eph family (Bennett *et al J. Biol. Chem.* 269. 14211-14218. 1994). The recruitment of Grb14 by Htk is of interest for two reasons. First, the expression profile of both Htk and the murine homologue myk-1 are indicative of a potential role in mammary gland development and neoplasia (Andres *et al Oncogene* 9. 1461-1467. 1994: Berclaz *et al Biochem. Biophys. Res. Comm.* 226. 869-875. 1996). Second, Eph family members may be involved in the regulation of cell migration (Tessier-Lavigne. *Cell* 82. 345-348. 1995), which is intriguing given the homology of the Grb7 family to the *C. elegans* protein mig10 (Stein *et al.* 1994 *supra*).

A novel cDNA of 1971 bp, designated 2.2412, was also isolated. This clone encoded a polypeptide of 657 amino acids in frame with the Gal4 DNA-BD. The cDNA did not contain a stop codon, and this, together with the Northern analysis described below, indicated that it was incomplete. This DNA fragment was therefore used as a probe to screen a human placental cDNA library (5' STRETCH PLUS, Clontech, in λgt10). This resulted in the isolation of two clones, designated clone 8 and clone 12. Clone 8 was approximately 2 kb and overlapped the original 2.2412 clone by 900 bp at the 3' end. This clone provided the carboxy-terminal end of the 2.2412 protein sequence (Figure 1). Clone 12 was approximately 3.5 kb and to date has provided an additional 692 bp of sequence information in the 5' direction. The nucleotide and protein sequence for 2.2412 provided by these overlapping clones is shown in Figure 1. Since a 5' initiation codon has yet to be identified the coding sequence still appears to be incomplete.

Further characterization of 2.2412

Database searches using the 2.2412 cDNA sequence revealed significant homology with a large number of proteins containing ankyrin-like repeats. These sequences were first identified as homologous regions between certain cell cycle regulatory proteins and the Drosophila protein Notch (Breeden and Nasmyth, *Nature* 329, 651-654, 1987) but subsequently they have been identified in a wide variety of other proteins where they are thought to function in protein-protein interactions (Bork, *Proteins* 17, 363-374, 1993). Subsequent analysis of the protein sequence identified 18 consecutive ankyrin repeats and an additional repetitive element (Figure 1). The ankyrin repeat region is followed by a stretch of approximately 40 amino

acids rich in serine residues. The remaining C-terminal region has a relatively high content of charged amino acids.

Northern analysis of 2.2412 mRNA expression

Northern blot analysis of multiple tissue northerns (Clontech) was performed using the original 2.2412 cDNA as a probe. This resulted in the detection of a single mRNA transcript of approximately 7 kb in all tissues examined with the exception of the kidney. Expression was particularly high in skeletal muscle and placenta. The size of this transcript compared to that of the 2.2412 clone indicates that the latter represents only a partial cDNA.

Genomic localization of the 2.2412 gene

Fluorescence *in situ* hybridization of the original 2.2412 cDNA to normal metaphases (Baker *et al*, 1996 *supra*) and reference to the FRA10A fragile site at 10q23.32 localized the gene to between chromosome 10q23.2 and proximal 10q23.32. Interestingly, deletions in the 10q22-25 region of chromosome 10 have been detected in a variety of human cancers including breast, prostate, renal, small cell lung and endometrial carcinomas, glioblastoma multiforme, melanoma and meningiomas, suggesting the presence of one or more tumour suppressive loci in this region (Li *et al*, *Science* 275, 1943-1947, 1997; Steck *et al*, *Nature Genetics* 15, 356-362, 1997, and references therein). Two candidate tumour suppressor genes have been identified in this region (MMAC1/PTEN and MXI1, Li *et al* 1997 *supra*; Steck *et al* 1997 *supra*; Albarosa *et al*, *Hum. Genet.* 95, 709-711, 1995).

Analysis of the interaction between 2.2412 and Grb7 family members

cDNAs encoding the full length and N- and C-terminal regions of the original 2.2412 cDNA clone (nucleotides 694-2664, 694-1614 and 1615-2664 of the sequence shown in Figure 1, respectively) were cloned into the vector pGEX4T2 (Pharmacia). The full length construct was generated by subcloning from the pACT2 clone as a NdeI fragment, whereas the shorter constructs were synthesized by directional cloning of PCR products. The corresponding GST-fusion proteins were purified from IPTG-induced bacterial cultures using glutathione-agarose beads (Smith and Johnson, *Gene* 67, 31-40, 1988). These immobilized fusion proteins were then incubated with lysates from cells expressing Flag epitope-tagged Grb14 (Daly *et al*, 1996

supra) or human breast cancer cells expressing high levels of Grb7 (SK-BR-3: Stein *et al.* 1994) as described previously (Daly *et al.* 1996). Following washing, bound proteins were detected by Western blot analysis. The results indicated that 2.2412 bound specifically to both Grb14 and Grb7 *in vitro*, and that the N-terminal fusion protein bound more strongly than that derived from the C-terminus. These data, obtained using a different methodology for detecting protein-protein interactions to the yeast two hybrid system, confirm that 2.2412 interacts with Grb14. Furthermore, 2.2412 also binds Grb7. Consequently 2.2412 appears to represent a general effector for the Grb7 family.

Mapping of the 2.2412 binding region on Grb14

In order to identify the region of Grb14 that interacts with 2.2412, a series of Grb14 deletion mutants were generated by cloning PCR fragments synthesized using the appropriate flanking primers into the vector pAS2.1. These fragments spanned the following regions: N-terminus ("N", amino acids 1-110), the central region ("C") encompassing the mig10 homology and the "between PH and SH2" (BPS) domain (amino acids 110-437) and the N-terminal and central regions ("N + C", amino acids 1-437). These plasmids were individually transformed into the yeast strain Y190 (*MATa, ura3-52, his3-200, ade2-101, lys2-801, trp1-901, leu2-3, 112, gal4Δ, gal80Δ, cyh^{r}2, LYS2::GAL1$_{UAS}$-HIS3$_{TATA}$-HIS3, URA3::GAL1$_{UAS}$-GAL1$_{TATA}$-lacZ*) and expression of the appropriately sized Gal4 DNA-BD fusion proteins confirmed by Western blotting. Following transformation of the resulting yeast strains with the original 2.2412 cDNA clone in pACT-2, the strength of the interaction was determined by either liquid- or filter-based β-galactosidase assays. The results are presented in Figure 2, and demonstrate that the N-terminal region of Grb14 is not only required, but is also sufficient, for binding 2.2412. This supports the hypothesis that 2.2412 represents a general effector for the Grb7 family, since the N-terminal region of these proteins contains a highly conserved proline-rich motif which may mediate this interaction.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Sequence listings:

SEQUENCE LISTING

Applicant: Garvan Institute of Medical Research

Title of Invention: A potential effector for the Grb7 family of signalling proteins.

Current Application Number:
Current Filing Date:

Prior Application Number:PO9388
Prior Application Filing Date: 1997-09-23

Number of ID SEQ Nos: 2

Software: PatentIn Ver. 2.0

SEQ ID NO: 1
Length: 3400
Type: DNA
Organism: Homo sapiens

Sequence: 1
attcctcttc ataatgcatg ctcttttggt catgctgaag tagtcaatct ccttttgcga 60
catggtgcag accccaatgc tcgagataat tggaattata ctcctctcca tgaagctgca 120
attaaaggaa agattgatgt ttgcattgtg ctgttacagc atggagctga gccaaccatc 180
cgaaatacag atggaaggac agcattggat ttagcagatc catctgccaa agcagtgctt 240
actggtgaat ataagaaaga tgaactctta gaaagtgcca ggagtggcaa tgaagaaaaa 300
atgatggctc tactcacacc attaaatgtc aactgccacg caagtgatgg cagaaagtca 360
actccattac atttggcagc aggatataac agagtaaaga ttgtacagct gttactgcaa 420
catggacgtg atgtccatgc taaagataaa ggtgatctgg taccattaca caatgcctgt 480
tcttatggtc attatgaagt aactgaactt ttggtcaagc atggtggctg tgtaaatgca 540
atggacttgt ggcaattcac tcctcttcat gaggcagctt ctaagaacag ggttgaagta 600
tgttctcttc tcttaagtta tggtgcagac ccaacactgc tcaattgtaa gaataaaagt 660
gctatagact tggctcccac accacagtta aaagaaagat tagcatatga atttaaaggc 720
cactcgttgc tgcaagctgc acgagaagct gatgttactc gaatcaaaaa acatctctct 780
ctggaaatgg tgaatttcaa gcatcctcaa acacatgaaa cagcattgca ttgtgctgct 840
gcatctccat atcccaaaag aaagcaaata tgtgaactgt tgctaagaaa aggagcaaac 900
atcaatgaaa agactaaaga attcttgact cctctgcacg tggcatctga gaaagctcat 960
aatgatgttg ttgaagtagt ggtgaaacat gaagcaaagg ttaatgctct ggataatctt 1020
ggtcagactt ctctacacag agctgcatat tgtggtcatc tacaaacctg ccgcctactc 1080
ctgagctatg ggtgtgatcc taacattata tcccttcagg gctttactgc tttacagatg 1140
ggaaatgaaa atgtacagca actcctccaa gagggtatct cattaggtaa ttcagaggca 1200
gacagacaat tgctggaagc tgcaaaggct ggagatgtcg aaactgtaaa aaaactgtgt 1260
actgttcaga gtgtcaactg cagagacatt gaagggcgtc agtctacacc acttcatttt 1320
gcagctgggt ataacagagt gtccgtggtg gaatatctgc tacagcatgg agctgatgtg 1380
catgctaaag ataaaggagg ccttgtacct ttgcacaatg catgttcttt cggacattat 1440
gaagttgcag aacttcttgt taaacatgga gcagtagtta atgtagctga ttatggaaa 1500
tttacacctt tacatgaagc agcagcaaaa ggaaaatatg aaatttgcaa acttctgctc 1560
cagcatggtg cagaccctac aaaaaaaaac agggatggaa atactccttt ggatcttgtt 1620
aaagatggag atacagatat tcaagatctg cttaggggag atgcagcttt gctagatgct 1680
gccaagaagg gttgtttagc cagagtgaag aagttgtctt ctcctgataa tgtaaattgc 1740
cgcgataccc aaggcagaca ttcaacacct ttacatttag cagctggtta taataattta 1800
gaagttgcag agtatttgtt acaacacgga gctgatgtga atgcccaaga caaaggagga 1860
cttattcctt tacataatgc agcatcttac gggcatgtag atgtagcagc tctactaata 1920

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aagtataatg catctctcaa tgccacggac aaatgggctt tcacacctt gcacgaagca 1980
gcccaaaagg gacgaacaca gctttgtgct ttgttgctag cccatggaqc tgacccgact 2040
cttaaaaatc aggaaggaca aacaccttta gatttagttt cagcagatga tgtcagcgct 2100
cttctgacag cagccatgcc cccatctgct ctgccctctt gttacaagcc tcaagtgctc 2160
aatggtgtga gaagcccagg agccactgca gatgctctct cttcaggtcc atctagccca 2220
tcaagccttt ctgcagccag cagtcttgac aacttatctg ggagtttttc agaactgtct 2280
tcagtagtta gttcaagtgg aacagagggt gcttccagtt tggagaaaaa ggaggttcca 2340
ggagtagatt ttagcataac tcaattcgta aggaatcttg gacttgagca cctaatggat 2400
atatttgaga gagaacagat cactttggat gtattagttg agatgggaca caaggagctg 2460
aaggagattg gaatcaatgc ttatggacat aggcacaaac taattaaagg agtcgagaga 2520
cttatctccg gacaacaagg tcttaaccca tatttaactt tgaacacctc tggtagtgga 2580
acaattctta tagatctgtc tcctgatgat aaagagtttc agtctgtgga ggaagagatg 2640
caaagtacag ttcgagagca cagagatgga ggtcatgcag gtggaatctt caacagatac 2700
aatattctca agattcagaa ggtttgtaac aagaaactat gggaaagata cactcaccgg 2760
agaaaagaag tttctgaaga aaaccacaac catgccaatg aacgaatgct atttcatggg 2820
tctccttttg tgaatgcaat tatccacaaa ggctttgatg aaaggcatac gtacataggt 2880
ggtatgtttg gagctggcat ttattttgct gaaaactctt ccaaaagcaa tcaatatgta 2940
tatggaattg gaggaggtac tgggtgtcca gttcacaaag acagatcttg ttacatttgc 3000
cacaggcagc tgctcttttg ccgggtaacc ttgggaaagt ctttcctgca gttcagtgca 3060
atgaaaatgg cacattctcc tccaggtcat cactcagtca ctggtaggcc cagtgtaaat 3120
ggcctagcat tagctgaata tgttatttac agaggagaac aggcttatcc tgagtattta 3180
attacttacc agattatgag gcctgaaggt atggtcgatg gataaatagt tattttaaga 3240
aactaattcc actgaaccta aaatcatcaa agcagcagtg gcctctacgt tttactcctt 3300
tgctgaaaaa aaatcatctt gcccacaggc ctgtggcaaa aggataaaaa tgtgaacgaa 3360
gtttaacatt ctgacttgat aaagctttaa taatgtacag                      3400
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SEQ ID NO: 2
Length: 1074
Type: PRT
Organism: Homo sapiens

Sequence: 2

```
Ile Pro Leu His Asn Ala Cys Ser Phe Gly His Ala Glu Val Val Asn
  1               5                  10                  15

Leu Leu Leu Arg His Gly Ala Asp Pro Asn Ala Arg Asp Asn Trp Asn
            20                  25                  30

Tyr Thr Pro Leu His Glu Ala Ala Ile Lys Gly Lys Ile Asp Val Cys
        35                  40                  45

Ile Val Leu Leu Gln His Gly Ala Glu Pro Thr Ile Arg Asn Thr Asp
    50                  55                  60

Gly Arg Thr Ala Leu Asp Leu Ala Asp Pro Ser Ala Lys Ala Val Leu
65                  70                  75                  80

Thr Gly Glu Tyr Lys Lys Asp Glu Leu Leu Glu Ser Ala Arg Ser Gly
                85                  90                  95

Asn Glu Glu Lys Met Met Ala Leu Leu Thr Pro Leu Asn Val Asn Cys
            100                 105                 110

His Ala Ser Asp Gly Arg Lys Ser Thr Pro Leu His Leu Ala Ala Gly
        115                 120                 125

Tyr Asn Arg Val Lys Ile Val Gln Leu Leu Leu Gln His Gly Arg Asp
    130                 135                 140

Val His Ala Lys Asp Lys Gly Asp Leu Val Pro Leu His Asn Ala Cys
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145                 150                 155                 160

Ser Tyr Gly His Tyr Glu Val Thr Glu Leu Leu Val Lys His Gly Gly
                165                 170                 175

Cys Val Asn Ala Met Asp Leu Trp Gln Phe Thr Pro Leu His Glu Ala
            180                 185                 190

Ala Ser Lys Asn Arg Val Glu Val Cys Ser Leu Leu Leu Ser Tyr Gly
        195                 200                 205

Ala Asp Pro Thr Leu Leu Asn Cys Lys Asn Lys Ser Ala Ile Asp Leu
    210                 215                 220

Ala Pro Thr Pro Gln Leu Lys Glu Arg Leu Ala Tyr Glu Phe Lys Gly
225                 230                 235                 240

His Ser Leu Leu Gln Ala Ala Arg Glu Ala Asp Val Thr Arg Ile Lys
                245                 250                 255

Lys His Leu Ser Leu Glu Met Val Asn Phe Lys His Pro Gln Thr His
            260                 265                 270

Glu Thr Ala Leu His Cys Ala Ala Ala Ser Pro Tyr Pro Lys Arg Lys
        275                 280                 285

Gln Ile Cys Glu Leu Leu Leu Arg Lys Gly Ala Asn Ile Asn Glu Lys
    290                 295                 300

Thr Lys Glu Phe Leu Thr Pro Leu His Val Ala Ser Glu Lys Ala His
305                 310                 315                 320

Asn Asp Val Val Glu Val Val Val Lys His Glu Ala Lys Val Asn Ala
                325                 330                 335

Leu Asp Asn Leu Gly Gln Thr Ser Leu His Arg Ala Ala Tyr Cys Gly
            340                 345                 350

His Leu Gln Thr Cys Arg Leu Leu Leu Ser Tyr Gly Cys Asp Pro Asn
        355                 360                 365

Ile Ile Ser Leu Gln Gly Phe Thr Ala Leu Gln Met Gly Asn Glu Asn
    370                 375                 380

Val Gln Gln Leu Leu Gln Glu Gly Ile Ser Leu Gly Asn Ser Glu Ala
385                 390                 395                 400

Asp Arg Gln Leu Leu Glu Ala Ala Lys Ala Gly Asp Val Glu Thr Val
                405                 410                 415

Lys Lys Leu Cys Thr Val Gln Ser Val Asn Cys Arg Asp Ile Glu Gly
            420                 425                 430

Arg Gln Ser Thr Pro Leu His Phe Ala Ala Gly Tyr Asn Arg Val Ser
        435                 440                 445

Val Val Glu Tyr Leu Leu Gln His Gly Ala Asp Val His Ala Lys Asp
    450                 455                 460

Lys Gly Gly Leu Val Pro Leu His Asn Ala Cys Ser Tyr Gly His Tyr
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465                 470                 475                 480

Glu Val Ala Glu Leu Leu Val Lys His Gly Ala Val Val Asn Val Ala
                485                 490                 495

Asp Leu Trp Lys Phe Thr Pro Leu His Glu Ala Ala Ala Lys Gly Lys
            500                 505                 510

Tyr Glu Ile Cys Lys Leu Leu Leu Gln His Gly Ala Asp Pro Thr Lys
        515                 520                 525

Lys Asn Arg Asp Gly Asn Thr Pro Leu Asp Leu Val Lys Asp Gly Asp
    530                 535                 540

Thr Asp Ile Gln Asp Leu Leu Arg Gly Asp Ala Ala Leu Leu Asp Ala
545                 550                 555                 560

Ala Lys Lys Gly Cys Leu Ala Arg Val Lys Lys Leu Ser Ser Pro Asp
                565                 570                 575

Asn Val Asn Cys Arg Asp Thr Gln Gly Arg His Ser Thr Pro Leu His
            580                 585                 590

Leu Ala Ala Gly Tyr Asn Asn Leu Glu Val Ala Glu Tyr Leu Leu Gln
        595                 600                 605

His Gly Ala Asp Val Asn Ala Gln Asp Lys Gly Gly Leu Ile Pro Leu
    610                 615                 620

His Asn Ala Ala Ser Tyr Gly His Val Asp Val Ala Ala Leu Leu Ile
625                 630                 635                 640

Lys Tyr Asn Ala Ser Leu Asn Ala Thr Asp Lys Trp Ala Phe Thr Pro
                645                 650                 655

Leu His Glu Ala Ala Gln Lys Gly Arg Thr Gln Leu Cys Ala Leu Leu
            660                 665                 670

Leu Ala His Gly Ala Asp Pro Thr Leu Lys Asn Gln Glu Gly Gln Thr
        675                 680                 685

Pro Leu Asp Leu Val Ser Ala Asp Asp Val Ser Ala Leu Leu Thr Ala
    690                 695                 700

Ala Met Pro Pro Ser Ala Leu Pro Ser Cys Tyr Lys Pro Gln Val Leu
705                 710                 715                 720

Asn Gly Val Arg Ser Pro Gly Ala Thr Ala Asp Ala Leu Ser Ser Gly
                725                 730                 735

Pro Ser Ser Pro Ser Ser Leu Ser Ala Ala Ser Ser Leu Asp Asn Leu
            740                 745                 750

Ser Gly Ser Phe Ser Glu Leu Ser Ser Val Val Ser Ser Ser Gly Thr
        755                 760                 765

Glu Gly Ala Ser Ser Leu Glu Lys Lys Glu Val Pro Gly Val Asp Phe
    770                 775                 780

Ser Ile Thr Gln Phe Val Arg Asn Leu Gly Leu Glu His Leu Met Asp
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785 790 795 800

Ile Phe Glu Arg Glu Gln Ile Thr Leu Asp Val Leu Val Glu Met Gly
805 810 815

His Lys Glu Leu Lys Glu Ile Gly Ile Asn Ala Tyr Gly His Arg His
820 825 830

Lys Leu Ile Lys Gly Val Glu Arg Leu Ile Ser Gly Gln Gln Gly Leu
835 840 845

Asn Pro Tyr Leu Thr Leu Asn Thr Ser Gly Ser Gly Thr Ile Leu Ile
850 855 860

Asp Leu Ser Pro Asp Asp Lys Glu Phe Gln Ser Val Glu Glu Glu Met
865 870 875 880

Gln Ser Thr Val Arg Glu His Arg Asp Gly Gly His Ala Gly Gly Ile
885 890 895

Phe Asn Arg Tyr Asn Ile Leu Lys Ile Gln Lys Val Cys Asn Lys Lys
900 905 910

Leu Trp Glu Arg Tyr Thr His Arg Arg Lys Glu Val Ser Glu Glu Asn
915 920 925

His Asn His Ala Asn Glu Arg Met Leu Phe His Gly Ser Pro Phe Val
930 935 940

Asn Ala Ile Ile His Lys Gly Phe Asp Glu Arg His Ala Tyr Ile Gly
945 950 955 960

Gly Met Phe Gly Ala Gly Ile Tyr Phe Ala Glu Asn Ser Ser Lys Ser
965 970 975

Asn Gln Tyr Val Tyr Gly Ile Gly Gly Gly Thr Gly Cys Pro Val His
980 985 990

Lys Asp Arg Ser Cys Tyr Ile Cys His Arg Gln Leu Leu Phe Cys Arg
995 1000 1005

Val Thr Leu Gly Lys Ser Phe Leu Gln Phe Ser Ala Met Lys Met Ala
1010 1015 1020

His Ser Pro Pro Gly His His Ser Val Thr Gly Arg Pro Ser Val Asn
1025 1030 1035 1040

Gly Leu Ala Leu Ala Glu Tyr Val Ile Tyr Arg Gly Glu Gln Ala Tyr
1045 1050 1055

Pro Glu Tyr Leu Ile Thr Tyr Gln Ile Met Arg Pro Glu Gly Met Val
1060 1065 1070

Asp Gly

Claims:

1. An isolated polynucleotide molecule encoding a candidate effector protein for the Grb7 family of signalling proteins, wherein the polynucleotide molecule comprises a nucleotide sequence having at least 75% sequence identity to that shown as SEQ ID NO: 1.

2. A polynucleotide molecule according to claim 1, wherein the polynucleotide molecule comprises a nucleotide sequence having at least 85% sequence identity to that shown as SEQ ID NO: 1.

3. A polynucleotide molecule according to claim 1, wherein the polynucleotide molecule comprises a nucleotide sequence having at least 95% sequence identity to that shown as SEQ ID NO: 1.

4. A polynucleotide molecule according to claim 1, wherein the polynucleotide molecule comprises a nucleotide sequence which substantially corresponds to that shown as SEQ ID NO: 1.

5. A host cell transformed with a polynucleotide molecule according to any one of the preceding claims.

6. A host cell according to claim 5, wherein the host cell is a mammalian, insect, yeast or bacterial host cell.

7. A method of producing a protein, comprising culturing the host cell of claim 5 or 6 under conditions suitable for the expression of the polynucleotide molecule and optionally recovering the protein.

8. A purified protein encoded by a polynucleotide molecule according to any one of claims 1 to 4.

9. A purified protein according to claim 8, wherein the protein comprises an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 2.

10. A fusion protein comprising an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 2.

11. An antibody or fragment thereof which specifically binds to a protein according to claim 8 or 9.

12. An oligonucleotide probe comprising a nucleotide sequence of at least 12 nucleotides, the oligonucleotide probe comprising a nucleotide sequence such that the oligonucleotide probe selectively hybridises to the polynucleotide molecule of any one of claims 1 to 4 under high stringency conditions.

13. An oligonucleotide probe according to claim 12, wherein the oligonucleotide probe comprises a nucleotide sequence of at least 18 nucleotides.

14. A method of detecting in a sample the presence of an effector protein for the Grb7 family of proteins, the method comprising reacting the sample with an antibody or fragment thereof according to claim 11.

15. A method of detecting in a sample the presence of mRNA encoding an effector protein for the Grb7 family of proteins, the method comprising reacting the sample with an oligonucleotide probe of claim 12 or 13.

FIGURE 1

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ATTCCTCTTCATAATGCATGCTCTTTTGGTCATGCTGAAGTAGTCAATCTCCTTTTGCGACATGGTGCAG   70
 I  P  L  H  N  A  C  S  F  G  H  A  E  V  V  N  L  L  R  H  G  A

ACCCCAATGCTCGAGATAATTGGAATTATACTCCTCTCCATGAAGCTGCAATTAAAGGAAAGATTGATGT  140
D  P  N  A  R  D  N  W  N  Y  T  P  L  H  E  A  A  I  K  G  K  I  D  V

TTGCATTGTGCTGTTACAGCATGGAGCTGAGCCAACCATCCGAAATACAGATGGAAGGACAGCATTGGAT  210
 C  I  V  L  L  Q  H  G  A  E  P  T  I  R  N  T  D  G  R  T  A  L  D

TTAGCAGATCCATCTGCCAAAGCAGTGCTTACTGGTGAATATAAGAAAGATGAACTCTTAGAAAGTGCCA  280
 L  A  D  P  S  A  K  A  V  L  T  G  E  Y  K  K  D  E  L  L  E  S  A

GGAGTGGCAATGAAGAAAAAATGATGGCTCTACTCACACCATTAAATGTCAACTGCCACGCAAGTGATGG  350
R  S  G  N  E  E  K  M  M  A  L  L  T  P  L  N  V  N  C  H  A  S  D  G

CAGAAAGTCAACTCCATTACATTTGGCAGCAGGATATAACAGAGTAAAGATTGTACAGCTGTTACTGCAA  420
 R  K  S  T  P  L  H  L  A  A  G  Y  N  R  V  K  I  V  Q  L  L  L  Q

CATGGACGTGATGTCCATGCTAAAGATAAAGGTGATCTGGTACCATTACACAATGCCTGTTCTTATGGTC  490
 H  G  R  D  V  H  A  K  D  K  G  D  L  V  P  L  H  N  A  C  S  Y  G

ATTATGAAGTAACTGAACTTTTGGTCAAGCATGGTGGCTGTGTAAATGCAATGGACTTGTGGCAATTCAC  560
H  Y  E  V  T  E  L  L  V  K  H  G  G  C  V  N  A  M  D  L  W  Q  F  T

TCCTCTTCATGAGGCAGCTTCTAAGAACAGGGTTGAAGTATGTTCTCTTCTCTTAAGTTATGGTGCAGAC  630
 P  L  H  E  A  A  S  K  N  R  V  E  V  C  S  L  L  L  S  Y  G  A  D

CCAACACTGCTCAATTGTAAGAATAAAAGTGCTATAGACTTGGCTCCCACACCACAGTTAAAAGAAAGAT  700
 P  T  L  L  N  C  K  N  K  S  A  I  D  L  A  P  T  P  Q  L  K  E  R

TAGCATATGAATTTAAAGGCCACTCGTTGCTGCAAGCTGCACGAGAAGCTGATGTTACTCGAATCAAAAA  770
L  A  Y  E  F  K  G  H  S  L  L  Q  A  A  R  E  A  D  V  T  R  I  K  K

ACATCTCTCTCTGGAAATGGTGAATTTCAAGCATCCTCAAACACATGAAACAGCATTGCATTGTGCTGCT  840
 H  L  S  L  E  M  V  N  F  K  H  P  Q  T  H  E  T  A  L  H  C  A  A

GCATCTCCATATCCCAAAAGAAAGCAAATATGTGAACTGTTGCTAAGAAAAGGAGCAAACATCAATGAAA  910
 A  S  P  Y  P  K  R  K  Q  I  C  E  L  L  L  R  K  G  A  N  I  N  E

AGACTAAAGAATTCTTGACTCCTCTGCACGTGGCATCTGAGAAAGCTCATAATGATGTTGTTGAAGTAGT  980
K  T  K  E  F  L  T  P  L  H  V  A  S  E  K  A  H  N  D  V  V  E  V  V

GGTGAAACATGAAGCAAAGGTTAATGCTCTGGATAATCTTGGTCAGACTTCTCTACACAGAGCTGCATAT 1050
 V  K  H  E  A  K  V  N  A  L  D  N  L  G  Q  T  S  L  H  R  A  A  Y

TGTGGTCATCTACAAACCTGCCGCCCTACTCCTGAGCTATGGGTGTGATCCTAACATTATATCCCTTCAGG 1120
 C  G  H  L  Q  T  C  R  L  L  L  S  Y  G  C  D  P  N  I  I  S  L  Q

GCTTTACTGCTTTACAGATGGGAAATGAAAATGTACAGCAACTCCTCCAAGAGGGTATCTCATTAGGTAA 1190
G  F  T  A  L  Q  M  G  N  E  N  V  Q  Q  L  L  Q  E  G  I  S  L  G  N

TTCAGAGGCAGACAGACAATTGCTGGAAGCTGCAAAGGCTGGAGATGTCGAAACTGTAAAAAAACTGTGT 1260
 S  E  A  D  R  Q  L  L  E  A  A  K  A  G  D  V  E  T  V  K  K  L  C

ACTGTTCAGAGTGTCAACTGCAGAGACATTGAAGGGCGTCAGTCTACACCACTTCATTTTGCAGCTGGGT 1330
 T  V  Q  S  V  N  C  R  D  I  E  G  R  Q  S  T  P  L  H  F  A  A  G

ATAACAGAGTGTCCGTGGTGGAATATCTGCTACAGCATGGAGCTGATGTGCATGCTAAAGATAAAGGAGG 1400
Y  N  R  V  S  V  V  E  Y  L  L  Q  H  G  A  D  V  H  A  K  D  K  G  G

CCTTGTACCTTTGCACAATGCATGTTCTTACGGACATTATGAAGTTGCAGAACTTCTTGTTAAAACATGGA 1470
 L  V  P  L  H  N  A  C  S  Y  G  H  Y  E  V  A  E  L  L  V  K  H  G

GCAGTAGTTAATGTAGCTGATTTATGGAAATTTACACCTTTACATGAAGCAGCAGCAAAAGGAAAATATG 1540
 A  V  V  N  V  A  D  L  W  K  F  T  P  L  H  E  A  A  A  K  G  K  Y

AAATTTGCAAACTTCTGCTCCAGCATGGTGCAGACCCTACAAAAAAAAACAGGGATGGAAATACTCCTTT 1610
E  I  C  K  L  L  L  Q  H  G  A  D  P  T  K  K  N  R  D  G  N  T  P  L
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GGATCTTGTTAAAGATGGAGATACAGATATTCAAGATCTGCTTAGGGGAGATGCAGCTTTGCTAGATGCT  1680
  D  L  V  K  D  G  D  T  D  I  Q  D  L  L  R  G  D  A  A  L  L  D  A

GCCAAGAAGGGTTGTTTAGCCAGAGTGAAGAAGTTGTCTTCTCCTGATAATGTAAATTGCCGCGATACCC  1750
  A  K  K  G  C  L  A  R  V  K  K  L  S  S  P  D  N  V  N  C  R  D  T

AAGGCAGACATTCAACACCTTTACATTTAGCAGCTGGTTATAATAATTTAGAAGTTGCAGAGTATTTGTT  1820
  Q  G  R  H  S  T  P  L  H  L  A  A  G  Y  N  N  L  E  V  A  E  Y  L  L

ACAACACGGAGCTGATGTGAATGCCCAAGACAAAGGAGGACTTATTCCTTTACATAATGCAGCATCTTAC  1890
  Q  H  G  A  D  V  N  A  Q  D  K  G  G  L  I  P  L  H  N  A  A  S  Y

GGGCATGTAGATGTAGCAGCTCTACTAATAAAGTATAATGCATCTCTCAATGCCACGGACAAATGGGCTT  1960
  G  H  V  D  V  A  A  L  L  I  K  Y  N  A  S  L  N  A  T  D  K  W  A

TCACACCTTTGCACGAAGCAGCCCAAAAGGGACGAACACAGCTTTGTGCTTTGTTGCTAGCCCATGGAGC  2030
  F  T  P  L  H  E  A  A  Q  K  G  R  T  Q  L  C  A  L  L  A  H  G  A

TGACCCGACTCTTAAAAATCAGGAAGGACAAACACCTTTAGATTTAGTTTCAGCAGATGATGTCAGCGCT  2100
  D  P  T  L  K  N  Q  E  G  Q  T  P  L  D  L  V  S  A  D  D  V  S  A

CTTCTGACAGCAGCCATGCCCCCATCTGCTCTGCCCTCTTGTTACAAGCCTCAAGTGCTCAATGGTGTGA  2170
  L  L  T  A  A  M  P  P  S  A  L  P  S  C  Y  K  P  Q  V  L  N  G  V

GAAGCCCAGGAGCCACTGCAGATGCTCTCTCTTCAGGTCCATCTAGCCCATCAAGCCTTTCTGCAGCCAG  2240
  R  S  P  G  A  T  A  D  A  L  S  S  G  P  S  S  P  S  S  L  S  A  A  S

CAGTCTTGACAACTTATCTGGGAGTTTTTCAGAACTGTCTTCAGTAGTTAGTTCAAGTGGAACAGAGGGT  2310
  S  L  D  N  L  S  G  S  F  S  E  L  S  S  V  V  S  S  S  G  T  E  G

GCTTCCAGTTTGGAGAAAAAGGAGGTTCCAGGAGTAGATTTTAGCATAACTCAATTCGTAAGGAATCTTG  2380
  A  S  S  L  E  K  K  E  V  P  G  V  D  F  S  I  T  Q  F  V  R  N  L

GACTTGAGCACCTAATGGATATATTTGAGAGAGAACAGATCACTTTGGATGTATTAGTTGAGATGGGGCA  2450
  G  L  E  H  L  M  D  I  F  E  R  E  Q  I  T  L  D  V  L  V  E  M  G  H

CAAGGAGCTGAAGGAGATTGGAATCAATGCTTATGGACATAGGCACAAACTAATTAAAGGAGTCGAGAGA  2520
    K  E  L  K  E  I  G  I  N  A  Y  G  H  R  H  K  L  I  K  G  V  E  R

CTTATCTCCGGACAACAAGGTCTTAACCCATATTTAACTTTGAACACCTCTGGTAGTGGAACAATTCTTA  2590
    L  I  S  G  Q  Q  G  L  N  P  Y  L  T  L  N  T  S  G  S  G  T  I  L

TAGATCTGTCTCCTGATGATAAAGAGTTTCAGTCTGTGGAGGAAGAGATGCAAAGTACAGTTCGAGAGCA  2660
  I  D  L  S  P  D  D  K  E  F  Q  S  V  E  E  E  M  Q  S  T  V  R  E  H

CAGAGATGGAGGTCATGCAGGTGGAATCTTCAACAGATACAATATTCTCAAGATTCAGAAGGTTTGTAAC  2730
    R  D  G  G  H  A  G  G  I  F  N  R  Y  N  I  L  K  I  Q  K  V  C  N

AAGAAACTATGGGAAAGATACACTCACCGGAGAAAAGAAGTTTCTGAAGAAAACCACAACCATGCCAATG  2800
    K  K  L  W  E  R  Y  T  H  R  R  K  E  V  S  E  E  N  H  N  H  A  N

AACGAATGCTATTTCATGGGTCTCCTTTTGTGAATGCAATTATCCACAAAGGCTTTGATGAAAGGCATGC  2870
  E  R  M  L  F  H  G  S  P  F  V  N  A  I  I  H  K  G  F  D  E  R  H  A

GTACATAGGTGGTATGTTTGGAGCTGGCATTTATTTTGCTGAAAACTCTTCCAAAAGCAATCAATATGTA  2940
    Y  I  G  G  M  F  G  A  G  I  Y  F  A  E  N  S  S  K  S  N  Q  Y  V

TATGGAATTGGAGGAGGTACTGGGTGTCCAGTTCACAAAGACAGATCTTGTTACATTTGCCACAGGCAGC  3010
    Y  G  I  G  G  G  T  G  C  P  V  H  K  D  R  S  C  Y  I  C  H  R  Q

TGCTCTTTTGCCGGGTAACCTTGGGAAAGTCTTTCCTGCAGTTCAGTGCAATGAAAATGGCACATTCTCC  3080
  L  L  F  C  R  V  T  L  G  K  S  F  L  Q  F  S  A  M  K  M  A  H  S  P

TCCAGGTCATCACTCAGTCACTGGTAGGCCCAGTGTAAATGGCCTAGCATTAGCTGAATATGTTATTTAC  3150
    P  G  H  H  S  V  T  G  R  P  S  V  N  G  L  A  L  A  E  Y  V  I  Y

AGAGGAGAACAGGCTTATCCTGAGTATTTAATTACTTACCAGATTATGAGGCCTGAAGGTATGGTCGATG  3220
    R  G  E  Q  A  Y  P  E  Y  L  I  T  Y  Q  I  M  R  P  E  G  M  V  D

GATAAATAGTTATTTTAAGAAACTAATTCCACTGAACCTAAAATCATCAAAGCAGCAGTGGCCTCTACGT  3290
G  *
```
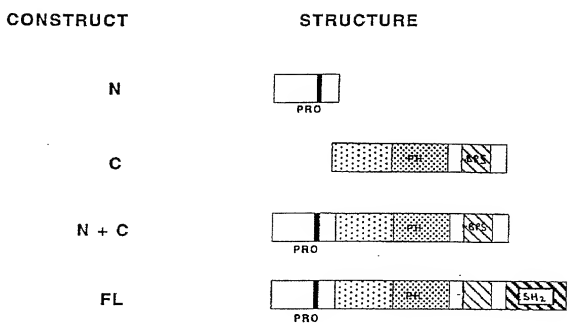

TTTACTCCTTTGCTGAAAAAAAATCATCTTGCCCACAGGCCTGTGGCAAAAGGATAAAAATGTGAACGAA 3360

GTTTAACATTCTGACTTGATAAAGCTTTAATAATGTACAG

A



B

CONSTRUCT	MEAN RLU (LIQUID ASSAY) (X 10^3)	COLOUR INTENSITY (FILTER ASSAY)
pAS2.1	4	-
N	109	++
C	3	-
N + C	194	++
FL	242	+++

FIGURE 2

DECLARATION, POWER OF ATTORNEY AND PETITION

As a below named inventor, I hereby declare that:

My residence, post office and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original first and joint inventor (if plural names are listed below) of the subject matter claimed and for which a patent is sought on the invention entitled:

A POTENTIAL EFFECTOR FOR THE GRB7 FAMILY OF SIGNALLING PROTEINS

the specification of which
☐ is attached hereto ☒ was filed on **23 September 1998** as Application No. **PCT/AU98/00795** and was amended on (if applicable).

I hereby state that I have reviewed and understand the consents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a)

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
PO 9388	Australia	23 September 1997	☒	☐
			☐	☐
			☐	☐
[Number]	[Country]	[Day/Month/Year Filed]	Yes	No

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

[Application Serial no]	[Filing Date]	[Status: patented, pending, abandoned]

I hereby declare that all statement s made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

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with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and all future correspondence should be addressed to them.
****

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../2

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